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Histochemical Muscle Fiber Characteristics of Texel Meat

Histochemické vlastnosti svalových vláken u jehňat plemene texel

Milena FANTOVÁ[1], Ludmila ŠKARKOVÁ[2], Martin PTÁČEK[1]*, Klára MICHNOVÁ[1], Lenka NOHEJLOVÁ[1] and Jaromír DUCHÁČEK[1]

[1] Department of Animal Husbandry, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamýcká 129, 165 21, Prague 6 - Suchdol, Czech Republic, *correspondence: ptacekm@af.czu.cz

[2] Department of Technological Equipment of Buildings, Faculty of Engineering, Czech University of Life Sciences, Kamýcká 129, 165 21, Prague 6 - Suchdol, Czech Republic

Abstract

The trial was performed on 12 Texel lambs (6 males and 6 females) in selected flock. The lambs were slaughtered at the age of 180 days and samples of *musculus longissimus lumborum et thoracis* (MLLT) and *musculus quadriceps femoris* (QFM) were collected. The histochemical traits of fiber cross sectional area, diameter and perimeter were monitored. Subsequently the fiber type distribution was calculated and evaluated. The dataset was evaluated using SAS. Significantly higher pH value (+0.88; $P < 0.01$) was marked in females, while significantly higher L* attribute was observed in males. No differences between males and females were detected in histochemical evaluation. On the other hand significant differences ($P < 0.05$ to 0.01) were observed in fiber type distribution and cross sectional area parameters of MLLT and QMF muscles. In praxis the results are usable for meat scientists, sheep breeders or the meat industry.

Keywords: cross sectional area, diameter, muscle fiber type distribution, musculus longissimus lumborum et thoracis, musculus quadriceps femoris, perimeter

Abstrakt

Studie byla provedena na 12 jehňatech plemene texel (6 beránků a 6 jehnic) ve vybraném chovu. U jehňat – poražených ve věku 180 dnech věku – byly odebrány vzorky svalů *musculus longissimus lumborum et thoracis* (MLLT) a *musculus quadriceps femoris* (QFM). Dále byly pozorovány histo-chemické ukazatele: plocha příčného řezu, průměr svalových vláken and jejich obvod. Následné byl dopočten a

vyhodnocen ukazatel zastoupení jednotlivých svalových vláken. Statistické vyhodnocení bylo provedeno v prgramu SAS. Statisticky významné rozdíly v závislosti na pohlaví jehňat nebyly pozorovány u histochemických ukazatelů svalových vláken. Na druhé straně průkazné rozdíly ($P < 0,05$ až $0,01$) byly zjištěny u zastoupení jednotlivých svalových vláken v mezi MLLT a QMF. Výsledky studie jsou využitelné pro vědeckou veřejnost, chovatele ovcí a pro masný průmysl.

Klíčová slova: musculus longissimus lumborum et thoracis, musculus quadriceps femoris, obvod, plocha příčného řezu, průměr, zastoupení svalových vláken

Introduction

The investigation of histochemical muscle fiber characteristics has practical importance to meat scientists, breeders, and the meat industry to provide a better understanding of the involvement of muscle fibers with regard to the determination of muscle growth and final meat quality traits (Wegner et al., 2000). As example relation histochemical characteristics on meat tenderness, water holding capacity, juiciness or fat content were published by (Čandek-Potokar et al., 1999; Lyczynski et al., 2009; Rehfeldt et al, 2000). The previous studies also confirmed influence of feed ration (Zabek et al., 2014), body part (Daniel et al., 2007; Yanar and Yetim, 2001), sex or genotype (Wegner et al., 2000) in different livestock muscle fiber characteristics. Anyway not many current studies focused on analysis histochemical characteristics of intensive meat purpose breed in extensive breeding conditions. The low-cost sheep management with permanent grazing pasture is preferred in the conditions of Czech Republic as well as in global conditions due to its maximal economic efficiency. The Texel is one of the most widespread sheep breed with perspective of using in this flock management system. Therefore the aim of this study was to evaluate the influence of sex and body part on physical and histochemical characteristics of Texel lamb meat.

Materials and methods

The study was performed at selected flock in the Central Bohemia region. The flock was situated at the altitude of 365 m above the sea level, with the average annual rainfall of 500 to 600 mm per year and average annual temperature of 8.5 °C. The animals were kept extensively all year-long outdoor with using natural shelters. The feed ration during the grazing season (April to September) was consisted of the grassland pasture and hay (ad libitum) only. There was no flushing effect applied before mating season. The sheep had access to mineral lick and to drinking water (ad libitum) during the whole year. In the non-grazing season, the ewes' feed ration consisted of hay (ad libitum). Approx. 6 weeks before lambing the haylage (3 kg per head per day) was added to ewes. The lambs had free choice access to mother's milk, grassland pasture, hay, drinking water and mineral licks. There was no concentrates supply for mothers and their lambs during the whole year.

Total of 12 Texel lambs (males, n = 6; females, n = 6) were selected from a flock of approx. 1000 ewes. All lambs (born and reared as twins) came from different mothers

at the age from 2 to 4 years. The lambs were born from April 1 till April 13 and their birth weight was 4.2 ± 0.3 kg. They were naturally reared together with the flock. At the age of 180 days the lambs were slaughtered according to EU laws.

Procedures were conducted according to the guidelines of the Council Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. The carcass weight (CW; ±0.1 kg) of lambs was assessed during 15 minutes after slaughter. At the same time the samples of *musculus longissimus lumborum et thoracis* (MLLT; n = 12) excesied at the area of 13th thoracis vertebrae and *musculus quadriceps femoris* (QFM; n = 12) excesied from the middle region of muscle (muscle length/2) were collected from the left side of the carcass for subsequent physical and histochemical analysis.

Samples were frozen in 2-methylbutane cooled by liquid nitrogen (-156 °C) and then stored at -80 °C until analysis. Cross-sections (12 µm) were cut with a cryostat Leica CM1850 (Leica Microsystems Nussloch GmbH, Nussloch, Germany) at -20 °C. Subsequently, staining for myofibrillar ATPase was performed after preincubation in alkaline buffer according to methodology by (Brooke and Kaiser, 1970). The types of muscle fibers were classified as type I (slow-twitch oxidative, slow oxidative, beta red, or red fibers), type IIA (fast-twitch oxidative, fast oxidative glycolytic, alpha red, or intermediate fibers), or as type IIB (fast-twitch glycolytic, fast glycolytic, alpha white, or white fibers) according to the nomenclature of previous authors, see Figure 1. Characteristics of muscle fibers (fiber cross sectional area – CSA in µm^2, diameter in µm, perimeter in µm) were determined using NIS Elements AR software (Version 3.2, 2006). Subsequently the fiber type distribution (FTD) was calculated.

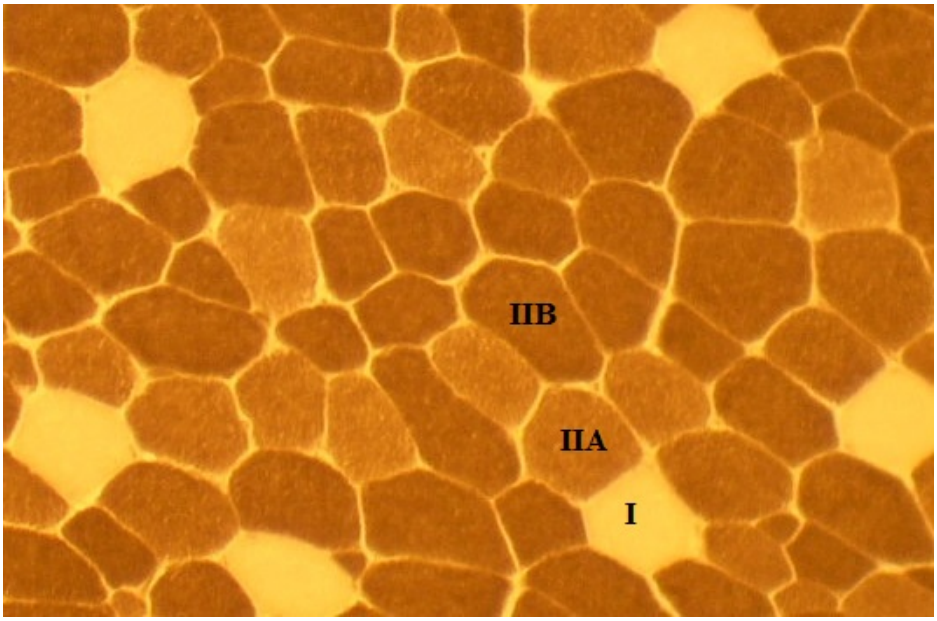

Figure 1. Muscle tissue fibers after preincubation in alkaline buffer. I = slow-twitch oxidative; IIA = fast-twitch oxidative-glycolytic; IIB = fast-twitch glycolytic (Brooke and Kaiser, 1970)
Obrázek 1. Svalová vlánka po preinkubaci v zásaditém pufru . I = pomalá vlákna oxidativní; IIA = rychlá vlákna oxido-glykotická; IIB = rychlá vlákna glykotická (Brooke and Kaiser, 1970)

Statistical analysis was carried out using SAS 9.3 (SAS/STAT® 9.3., 2011), UNIVARIATE and GLM (ANOVA) procedures. The fixed effects in model were sex of lambs (2 classes – males, n = 6; females, n = 6), sampling collection area (2 classes – MLLT, n = 12; QFM, n = 12). Regression on carcass weight was taken into the account. Tukey-Kramer method was applied for comparison and evaluation the significantly differences between least square means. Significance levels $P < 0.05$ and $P < 0.01$ were used to evaluate the differences between groups.

Results and discussion

The basic characteristics of dataset are presented in Table 1. The highest average CSA was detected in I muscle fiber (999.50 μm^2), while the lowest in IIA (736.58 μm^2). This tendency was observed also in diameter and perimeter characteristics. On the other hand, the lowest FTD was measured in I muscle fiber (11.38%), while the highest one in IIB (72.42%).

Table 1. Basic physical and histochemical characteristics of Texel lambs

Tabulka 1. Základní ukazatele fyzikálních a histochemických vlastností jehňat texel

Variable	n	$\bar{x}$	s	min.	max.	V (%)
CSA – total	24	877.78	236.35	508.29	1439.49	26.93
CSA – I	24	999.50	273.38	553.51	1753.63	27.35
CSA – IIA	24	736.58	178.90	425.35	1200.89	24.29
CSA – IIB	24	894.71	267.49	489.83	1601.05	29.90
DIA – I	24	34.45	4.61	26.38	44.34	13.38
DIA – IIA	24	29.51	3.58	22.39	38.16	12.13
DIA – IIB	24	31.81	4.64	23.71	42.90	14.59
PER – I	24	117.34	15.98	90.66	155.94	13.62
PER – IIA	24	104.84	12.00	79.59	133.83	11.45
PER – IIB	24	115.27	15.43	87.78	152.31	13.39
FTD – I	24	11.38	8.19	1.93	27.33	71.94
FTD – IIA	24	16.20	6.01	5.73	25.99	37.13
FTD – IIB	24	72.42	8.63	51.74	87.39	11.91

n = number of observing; $\bar{x}$ = aritmetic mean; s = standard deviation; min. = minimal value; max. = maximal value; V = variation coefficient; CSA – total = cross sectional area of all muscle fibers (μm^2); CSA – I = cross sectional area of slow slow-twitch oxidative muscle fiber (μm^2); CSA IIA = cross sectional area of fast-twitch oxidative-glycolytic muscle fiber (μm^2); CSA – IIB = cross sectional area of fast-twitch glycolytic muscle fiber (μm^2); DIA – I = diameter of slow slow-twitch oxidative muscle fiber (μm); DIA – IIA = diameter of fast-twitch oxidative-glycolytic muscle fiber (μm); DIA – IIB = diameter of fast-twitch glycolytic muscle fiber (μm); PER – I = perimeter of slow slow-twitch oxidative muscle fiber (μm); PER – IIA = perimeter of fast-twitch oxidative-glycolytic muscle fiber (μm); PER – IIB = perimeter of fast-twitch glycolytic muscle fiber (μm); FTD – I = fiber type distribution of slow slow-twitch oxidative muscle fiber (%); FTD – IIA = fiber type distribution of fast-twitch oxidative-glycolytic muscle fiber (%); FTD – IIB = fiber type distribution of fast-twitch glycolytic muscle fiber (%).

n = počet pozorování; $\bar{x}$ = aritmetický průměr; s = směrodatná odchylka; min. = minimální hodnota; max. = maximální hodnota; V = variační koeficient; CSA – total = plocha příčného řezu všech svalových vláken (μm^2); CSA – I = plocha pomalých svalových vláken oxidativních na příčném řezu (μm^2); CSA IIA = plocha rychlých svalových vláken oxido-glykotických na příčném řezu (μm^2); CSA – IIB = plocha rychlých svalových vláken glykotických na příčném řezu (μm^2); DIA – I = průměr pomalých svalových vláken oxidativních (μm); DIA – IIA = průměr rychlých svalových vláken oxido-glykotických (μm); DIA – IIB = diameter rychlých svalových vláken glykotických (μm); PER – I = obvod pomalých svalových vláken oxidativních (μm); PER – IIA = obvod rychlých svalových vláken oxido-glykotických (μm); PER – IIB = obvod rychlých svalových vláken glykotických (μm); FTD – I = zastoupení pomalých svalových vláken oxidativních (%); FTD – IIA = zastoupení rychlých svalových vláken oxido-glykotických (%); FTD – IIB = zastoupení rychlých svalových vláken glykotických (%).

Model used to evaluate histochemical characteristics was significant and it explained 11.5 to 53.7% variability of particular variables. Results of histochemical muscle fiber analysis performed on Texel lambs as well as significance of factors in model are presented in Table 2. No differences between males and females were detected in

this study, which was previously documented by non-significance of this factor in ANOVA evaluation. Results of presented study are in contrast to those of previously published by Fantová et al. (2015), Wojtysiak et al. (2010) or Velotto et al. (2010, 2005) on German Heath, Polish Longwool, Laticuada or Italian Merino lambs. Anyway, evenness of males vs. females could be explained by comparable growth intensity in specified natural condition and permanent grazing pasture management.

Suzuki (1971) as well as Suzuki and Cassens (1983) or Suzuki and Tamate (1988) did not find influence of sheep body part on muscle fibers size. Oppositely to their results significant differences between MLLT and QFM muscle were detected in presented study. Specifically, the higher CSA of total muscles fibers (+149.50 μm^2; $P < 0.05$) and IIB (+173.81 μm^2; $P < 0.05$) were found in QFM compared to MLLT. Similarly, lower perimeter of IIB muscle fiber (-8.86 μm; $P < 0.05$) was noticed in MLLT as well. Significantly higher FTD of I muscle fiber (+13.30%; $P < 0.01$) as well as significantly lower FTD of IIB (-10.27%; $P < 0.01$) was observed in QFM muscle compared to MLLT. Results from presented study are thus in large agreement with Fantová et al. (2015) and Daniel et al. (2007), who observed German Heat and Charollais crossbred lambs slaughtered at the age of 21 and 24 weeks. Peinado et al. (2004) investigated in detail the mean percentages or diameter of the muscle fibers types of the MLLT during the postnatal Segurena lamb's development. The distribution of particular fiber types in lambs at the age from 1 day to 90 days was as followed: 8.57 to 10.61% in I muscle fiber, 30.23 to 48.53% in IIA muscle fiber and 41.49 to 60.84% in IIB muscle fiber. Lower distribution of I and IIA muscles fibers, while the higher percentage distribution of IIB was observed in presented study. They added that the diameter of muscle fibers in MLLT was 10.45 to 25.20 μm of I fiber type, 5.62 to 13.30 μm of IIA and 6.59 to 21.65 μm of IIB fiber types. These results are in partial accordance when different letters but resemble rank of fiber types were detected also in Texel lambs. Similar values or similar rank of fiber type distribution could be connected with analogous physiological development of different sheep breeds. Differences in partial values could be explained by various carcass weight, slaughter age, breeding conditions or different lamb's genotype. Previous studies evaluated also muscle fibers in other livestock. Wegner et al. (2000) observed muscle fiber characteristics of semitendinosus muscle in four bovine genotypes (German Angus, Galloway and Belgian Blue breeds). Their results documented that highest FTD was observed in IIB muscle fiber (60 to 70%). Oppositely the lowest values were observed in I muscle fiber (7 to 15%). The results largely corresponded to those of presented study, confirming similarity of muscle fiber distribution in large and small meat ruminant.

Previous studies also confirmed close connection of histochemical muscle fiber characteristics and meat quality parameters. Čandek-Potokar et al. (1999) found that juiciness was positively correlated to CSA and fiber type distribution of IIB fibers, while mouth coating negatively. The negative influence of CSA parameter on water holding capacity and meat tenderness in pig meat was detected by Rehfeldt et al. (2000). Hawkins et al. (1985) suggested that the increase in the carcass fat content was related to an increase of I and IIA muscle fiber size. Based on above mentioned it seems that better meat quality characteristics were found in MLLT muscle, despite the detail sensory analysis was not performed. Therefore the results are usable for meat scientists, sheep breeders and the meat industry as well.

Table 2. Effect of sex of lambs and sample collection area on basic histochemical muscle fiber characteristics

Tabulka 2. Vliv pohlaví jehňat a oblasti místa odběru na základní histochemické ukazatele svalových vláken

		CSA (μm^2)				DIA (μm)			PER (μm)			FTD (%)		
		total	I	IIA	IIB	I	IIA	IIB	I	IIA	IIB	I	IIA	IIB
Sex	Males (n = 6)	791.34	871.55	700.23	787.96	32.31	28.77	30.16	110.17	104.03	108.96	10.70	16.35	72.95
	Females (n = 6)	964.22	1127.45	772.93	1001.47	36.59	30.25	33.46	124.50	105.65	121.57	12.06	16.04	71.89
	RMSE	62.531	86.166	58.583	71.902	1.477	1.195	1.309	4.987	3.752	4.054	1.881	2.146	2.730
Muscle	MLLT (n = 12)	803.03^a	993.87	722.33	807.81^a	34.39	29.37	30.68	116.97	104.83	110.84^a	4.73^A	17.71	77.55^A
	QFM (n = 12)	952.53^b	1005.13	750.83	981.62^b	34.51	29.65	32.94	117.70	104.85	119.70^b	18.03^B	14.68	67.28^B
	RMSE	47.522	65.484	44.521	54.644	1.122	0.909	0.995	3.790	2.852	3.081	1.429	1.631	2.075
Effects	Sex	0.118	0.094	0.471	0.094	0.103	0.470	0.152	0.105	0.801	0.081	0.672	0.933	0.821
	Muscle	0.038	0.905	0.656	0.037	0.942	0.832	0.125	0.893	0.997	0.056	<.0001	0.204	0.002
	CW	0.001	0.003	0.017	0.001	0.004	0.020	0.001	0.003	0.020	0.001	0.773	0.797	0.689

CSA – total = cross sectional area of all muscle fibers (μm^2); CSA – I = cross sectional area of slow slow-twitch oxidative muscle fiber (μm^2); CSA IIA = cross sectional area of fast-twitch oxidative-glycolytic muscle fiber (μm^2); CSA – IIB = cross sectional area of fast-twitch glycolytic muscle fiber (μm^2); DIA – I = diameter of slow slow-twitch oxidative muscle fiber (μm); DIA – IIA = diameter of fast-twitch oxidative-glycolytic muscle fiber (μm); DIA – IIB = diameter of fast-twitch glycolytic muscle fiber (μm); PER – I = perimeter of slow slow-twitch oxidative muscle fiber (μm); PER – IIA = perimeter of fast-twitch oxidative-glycolytic muscle fiber (μm); PER – IIB = perimeter of fast-twitch glycolytic muscle fiber (μm); FTD – I = fiber type distribution of slow slow-twitch oxidative muscle fiber (%); FTD – IIA = fiber type distribution of fast-twitch oxidative-glycolytic muscle fiber (%); FTD – IIB = fiber type distribution of fast-twitch glycolytic muscle fiber (%); MLLT - *musculus longissimus lumborum et thoracis*; QFM - *quadriceps femoris muscle*; CW - carcass weight of lambs; a-b or A-B different letters in columns means significant differences P < 0.05 or P < 0.01

CSA – total = plocha příčného řezu všech svalových vláken (μm^2); CSA – I = plocha pomalých svalových vláken oxidativních na příčném řezu (μm^2); CSA IIA = plocha rychlých svalových vláken oxido-glykotických na příčném řezu (μm^2); CSA – IIB = plocha rychlých svalových vláken glykotických na příčném řezu (μm^2); DIA – I = průměr pomalých svalových vláken oxidativních (μm); DIA – IIA = průměr rychlých svalových vláken oxido-glykotických (μm); DIA – IIB = diameter rychlých svalových vláken glykotických (μm); PER – I = obvod pomalých svalových vláken oxidativních (μm); PER – IIA = obvod rychlých svalových vláken oxido-glykotických (μm); PER – IIB = obvod rychlých svalových vláken glykotických (μm); FTD – I = zastoupení pomalých svalových vláken oxidativních (%); FTD – IIA = zastoupení rychlých svalových vláken oxido-glykotických (%); FTD – IIB = zastoupení rychlých svalových vláken glykotických (%); MLLT - *musculus longissimus lumborum et thoracis*; QFM – *musculus quadriceps femoris*; CW – porážková hmotnost jehňat; a-b nebo A-B odlišná písmena mezi sloupci znamenají průkazné rozdíly P < 0.05 nebo P < 0.01

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References

Brooke, M. H., Kaiser, K. K. (1970) Muscle fiber types: How many and what kind? Archives of Neurology, 23 (4), 369-379. DOI: 10.1001/archneur.1970.00480280083010

Council EEC (1986) EEC Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Official Journal of the European Communities, 29, L358.

Čandek-Potokar, M., Lefaucheur, L., Žlender, B., Bonneau, M. (1999) Effect of slaughter weight and/or age on histological characteristics of pig longissimus dorsi muscle as related to meat quality. Meat Science, 52 (2), 195-203. DOI: 10.1016/s0309-1740(98)00168-5

Daniel, Z. C. T. R., Brameld, J. M., Craigon, J., Scollan, N. D., Buttery, P. J. (2007) Effect of maternal dietary restriction during pregnancy on lamb carcass characteristics and muscle fiber composition. Journal of Animal Science, 85 (6), 1565-1576. DOI: 10.2527/jas.2006-743

Fantová, M., Ptáček, M., Michnová, K., Nohejlová, L., Ducháček, J. (2015) Histochemical muscle fibre characteristics of German Heath lamb meat. Acta Veterinaria Brno, 84 (3), 297-303. DOI: 10.2754/avb201584030297

Hawkins, R. R., Moody, W. G., Kemp, J. D. (1985) Influence of genetic type, slaughter weight and sex on ovine muscle fiber and fatcell development. Journal of Animal Science, 61 (5), 1154-1163.

Lyczynski, A., Runowska, G., Pospiech, E., Koćwin-Podsiadła, M., Wojtczak, J., Rzosinska, E., Grześ, B., Mikołajczak, B., Iwańska, E. (2009) Estimation of selected porcine meat quality indicators on the basis of electrical condictivity measured 24 hours post-slaughter. Animal Science Papers and Reports, 27 (1), 51-58.

Peinado, B., Latorre, R., Váquez-Autón, J. M., Poto, A., Ramírez, G., López-Albors, O., Moreno, F., Gil, F. (2004) Histochemical skeletal muscle fibre types in the sheep. Anatomia, Histologia, Embryologia, 33 (4), 236-243. DOI: 10.1111/j.1439-0264.2004.00545.x

Rehfeldt, C., Fiedler, I., Dietl, G., Ender, K. (2000) Myogenesis and postnatal skeletal muscle cell growth as influenced by selection. Livestock Production Science, 66 (2), 177-188. DOI: 10.1016/s0301-6226(00)00225-6

SAS (2011) SAS – STAT® 9.3. User’s Guide. Carry, NC: SAS Institute Inc.

Suzuki A. (1971) Histochemical classification of individual skeletal muscle fibers in the sheep. I. On M. semitendinosus, M. longissimus dorsi, M. psoas major, M. latissimus dorsi and M. gastrocnemius. Japaneese Journal of Zootechnical Science, 42 (1), 39-54. DOI: 10.2508/chikusan.42.39

Suzuki, A., Cassens, R. G. (1983) A histochemical study of myofiber types in the serratus ventralis thoracis muscle of sheep during growth. Journal of Animal Science, 56 (6), 1447-1458.

Suzuki, A., Tamate, H. (1988) Distribution of myofiber types in the hip and thight musculature in sheep. The Anatomical Record, 221 (1), 494-502. DOI: 10.1002/ar.1092210106

Velotto, S., Di Prisco, M. R., Stasi, T., Crasto, A. (2005) Histomorphometrical evaluation of myocyte types in the lamb. Acta Veterinaria Brno 74 (2), 175-182. DOI: 10.2754/avb200574020175

Velotto, S., Varricchio, E., Di Prisco, M. R., Stasi, T., Crasto, A. (2010) Effect of age and sex on histomorphometrical characteristics of two muscles of Laticuada lambs. Acta Veterinaria Brno, 79 (1), 3-12. DOI: 10.2754/avb201079010003

Wegner, J., Albrecht, E., Fiedler, I., Teuscher, F., Papstein, H. J., Ender, K. (2000) Growth – and breed – related changes of muscle fiber characteristics in cattle. Journal of Animal Science, 78 (6), 1485-1496.

Wojtysiak, D., Kaczor, U., Połtowicz, K., Krzysztoforski, K. (2010) The effects of sex and slaughter weight on muscle fibre characteristics and physico-chemical properties of lamb longissimus thoracis muscle. Animal Science Papers and Reports, 28 (1), 61-69

Yanar, M., Yetim, H. (2001) The effects of aging period and muscle type on the textural quality of characteristics of mutton. Turkish Journal of Veterinary and Animal Sciences, 25 (2), 203-207

Zabek, K., Milewski, S., Wójcik, R., Siwicki, A. K. (2014) The effects of supplementing diets fed to pregnant and lactating ewes with Saccharomyces cerevisiae dired yeast. Turkish Journal of Veterinary and Animal Sciences, 38 (2), 200-206. DOI: 10.3906/vet-1305-68